ELECTROKINETIC STUDIES OF MARINE OVA. VII. RELATION BETWEEN THE ZETA POTENTIAL AND ADHESIVENESS OF THE CELL MEMBRANE OF SEA-URCHIN EGGS

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In the field of colloid chemistry, the electrokinetic potential is a factor of paramount importance for the stabilization of suspensions and emulsions. In a suspension of a single substance the stability is minimum in the isoelectric condition.

Agglutination of living cells on abolishing the surface potential has been observed by many investigators in blood cells (Coulter, 1920, 1922; Northrop and Freund, 1924; Oliver and Barnard, 1925) and bacteria (Northrop and De Kruif, 1922a, 1922b; Eggerth, 1923; Shibley, 1924; Falk, 1928; Mudd, Nugent and Bullock, 1932; Mudd, 1933), and also in some plant cells (Pfeiffer, 1933, 1934). The object of the present paper is to see whether a similar relationship exists between potential and agglutination in such large cells as sea-urchin eggs and to investigate it from the standpoint of surface adhesiveness rather than of agglutination. In the majority of agglutination studies, it is conventional to give the critical potentials for agglutination. The critical potential however fails to give us adequate information about the physical state of the surface in the pre-agglutination states. The author thinks, for this reason, that the study of adhesiveness is better since it leads to a wider viewpoint. Studies of this kind are surprisingly few in the literature, presumably because of the inadequacy of methods of measuring adhesiveness (see Pfeiffer, 1935).

Method

In the experiments to be reported, the stream method was adopted. The simple glass apparatus which was constructed is diagrammatically shown in Figure 1. It consists of a series of tubes, each square in cross section but differing in dimension. The end with the largest bore is connected to a large flat reservoir (omitted from the figure). This end serves as the inlet, and at the opposite end, there is an outlet tube provided with a stop-cock. As is evident, for a single run of this outfit, five different fluid current strengths can be obtained, the current strengths being calculated by the amount of liquid flowing through the tube during a definite time interval. By combining a choice of different bores and different pressures, it is easy to get a sufficiently wide range of current strengths. Then by taking the percentages of remaining cells over the initial numbers for different current strengths, a curve can be drawn. This curve will be referred to as the "remainder-curve" in later paragraphs. The area circumscribed by the remainder curve and the two axes indicates the magnitude of the surface adhesiveness characteristic to the conditions under investigation.

The actual procedure of the experiment was as follows: a suspension of eggs of a suitable concentration was prepared, introduced into the inverted apparatus, and the eggs were allowed to settle and lie undisturbed for 10 minutes. (The interval of 10 minutes was arbitarily chosen but this interval was kept constant through all the experiments.) At the end of 10 minutes, the apparatus was reinverted (returned to the normal position), so that the eggs were now hanging from the ceiling, so to speak. Definite areas were then marked out along the midline of the tubes of each dimension, quite close to the outlet end, the total numbers of the cells included within the marked areas were counted, and the solution was allowed to flow for exactly 1 minute (interval arbitrarily chosen). The percentages of cells remaining after 1 minute's flow over the initial numbers in different current strengths give a remainder-curve. The positions of the marked-out areas were selected near the oulet end of each portion to avoid vortices in the flow near the inflow end caused by the narrowing of the tube. The distance from the inflow end necessary for obtaining a laminar flow is a function of the dimension of the tube and the current strength and can be calculated by the Reynolds number often used in hydronamics.

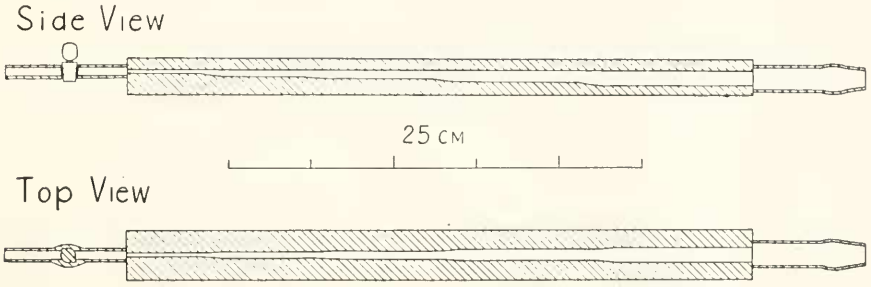

FIGURE 1. A diagram of the apparatus for measuring adhesiveness of the cell membrane. A series of tubes, square in cross section but differing in dimensions, is made through a glass bar (shaded). The large cylindrical tube on the right side is connected to a reservoir by rubber tubing. The small tube on the left side is an outlet and the current can be put on or shut off by the manipulation of the stop-cock. The eggs are made to adhere to the top wall in order to avoid the bumping of the washed-off cells into neighboring cells. It was desired to make the tube of the smallest dimension much longer, but this could not be realized because of a technical obstacle.

CRITICAL CONSIDERATION OF THE METHOD

Current strengths. In the data to be presented, the current strengths are calculated from the cross-sectional areas of the tubes and the amount of solution which flowed out during 1 minute. But, as is known, the current speed is not uniform throughout a cross-section, being maximal in the center and decreasing toward the walls. It is possible to treat this condition mathematically, but considering the other ambiguous terms involved such as 10 minutes' resting or 1 minute's streaming, the calculation was not attempted. Only the precaution was taken of selecting the positions of the marked-out areas along the midline of the top wall.

Turbulences. As was already mentioned, the object of marking out a definite area near the outlet end was to avoid the turbulences in the flow resulting from the narrowing of the tube. But this procedure could not eliminate the secondary turbu-

lences caused by the eggs themselves. In order to remove the errors of this source, care had to be taken to leave a certain distance between two adjacent eggs which could also be calculated. The distance necessary is the greater, the faster the current. As a matter of fact, if an adhering cell is observed by a microscope in a very strong current, the cell is sometimes seen to be vibrating, which will not happen unless it is being washed in a turbulent flow. Although no special attempt was made to remove errors of this kind, they were automatically minimized because of the fact that, in filling the apparatus with an egg suspension, the number of eggs which could get into a smaller tube, where the current speed was greater, was much less than that in a larger tube where the rate of flow was relatively slow.

Glass surface. The inner surfaces of the glass apparatus were polished in order to obtain as uniform a condition as possible and they were frequently cleaned with cleaning solution. But it soon came to the author's notice that the first reading after a cleaning was always higher than the succeeding readings. The readings from the second time on checked very well among each other. This is obviously due to the coating of the glass surfaces with proteins during the first measurement. For this reason, before starting a series of measurements, filling of the apparatus once with an egg suspension was practiced. Between successive measurements, the apparatus was washed with isotonic NaCl solution.

Limit of the method. The maximum current velocity tried in the present investigation was about 2m./sec. When the egg surface was made extremely adhesive by 1/200 M $CeCl_3$, even this flow speed could not wash off any cells. If microscopic observations were made of eggs in a current speed of around 2 m./sec., the eggs were seen to be deformed under the pressure exerted by the current. If the tearing force was further increased, finally the main bodies of the cells were torn off, leaving small portions of cytoplasm still adhering to the glass wall. This is not due to the failure of the membrane adhesiveness to resist the pressure but to the yielding of the membrane tension and the cohering force of the protoplasm to the force applied. This sets a limit to the method.

Results

Hydrogen ion concentrations and adhesiveness. During the work reported in a previous paper (Dan, 1947a), it was noted that when the concentration of hydrogen ions of the medium was increased, the sea-urchin eggs stuck together in various degrees roughly proportional to the concentration of hydrogen ions. In the literature, observations on the agglutination of sea-urchin eggs when an acid is added to sea water are frequently met with (Gray, 1916; Runnström, 1929; Vlès, 1924).

The present experiment was performed on the unfertilized eggs of *Anthocidaris crassispina* from which the jelly had previously been removed. The pH values taken were 8.2 (natural sea water), 6.9, 4.7 and 2.7, salt corrections being made by Clark's table. The cataphoretic potentials at these pH's are -23.0, -26.3, -18.5 and -11.0 millivolts respectively (Dan, 1947a). The reasons for selecting these pH values are that the first is the natural medium, the second is the medium in which the absolute magnitude of the potential is the largest, the third corresponds to a point which is just above the critical potential for flocculation of the cells and the last is a very acidic medium. The most acidic medium investigated in the

previous work (pH 2.0) was intentionally avoided for a reason which will be discussed below.

One of the typical results of the adhesiveness measurements is shown in Table 1 A and B and Figures 2 A and B. It is to be noticed that although variation does exist in the adhesiveness of different batches, the general feature is very consistent.

TABLE 1

The remainder percentages in media of different pH's

A	Stream method										Gravity method
Medium	%	cm./sec.	%	cm./sec.	%	cm./sec.	%	cm./sec.	%	cm./sec.	%
Sea water pH 8.2 (23.0 m.v.)	60.0	0.40	66.7	0.65	13.0	1.28	0	2.00	0*	8.00*	45.8
	49.1	0.40	37.5	0.65	16.7	1.28	3.8	2.00	0	8.00	67.0
Sea water pH 4.7 (−18.5 m.v.)	100.0*	0.41	100.0	0.68*	100.0*	1.33*	100.0*	2.08*	100.0*	8.33*	100.0
	100.0	0.58	100.0*	0.95*	100.0	1.87	100.0*	2.92*	100.0	11.53	100.0
	100.0*	3.74*	100.0*	6.19*	100.0	12.13	70.1	18.96	4.5	75.83	—
	100.0	3.89	100.0	6.43	83.8	12.60	45.3	19.69	0	78.75	100.0
	98.7	4.07	93.1	6.74	79.6	13.20	49.3	20.63	6.3	82.50	100.0
Sea water pH 2.7 (−11.0 m.v.)	—	—	100.0	6.80	98.6	13.33	93.7	20.83	81.5	83.33	100.0
	100.0	7.61	100.0	12.59	98.6	24.67	93.7	38.54	81.5	154.16	—
B	Stream method										Gravity method
Medium	%	cm./sec.	%	cm./sec.	%	cm./sec.	%	cm./sec.	%	cm./sec.	%
Sea water pH 8.2 (−23.0 m.v.)	94.7	0.35	88.3	0.58	67.1	1.13	44.3	1.79	9.5	7.08	7.6
	77.7	0.56	75.5	0.92	56.1	1.80	41.0	2.81	4.8	11.25	—
	100.0	0.33	98.1	0.54	84.9	1.07	73.3	1.67	3.7	6.67	4.9
Sea water pH 6.9 (−26.3 m.v.)	100.0*	0.33*	100.0*	0.55*	100.0*	1.08*	68.4	1.69	5.7	6.75	2.0
	100.0*	0.56*	100.0*	0.92*	100.0	1.80	65.3	2.81	2.9	11.25	—
	—	—	99.1*	0.54*	100.0*	1.07*	72.2	1.67	1.9	6.67	1.6
	—	—	99.1	0.95	98.1	1.86	54.6	2.91	1.9	11.67	—
Sea water pH 4.7 (−18.5 m.v.)	100.0*	0.37*	100.0*	0.61*	100.0*	1.20*	100.0*	1.87*	97.4	7.50	100.0
	100.0*	1.90*	100.0	3.13	100.0	6.13	100.0	9.59	57.9	38.33	—
	100.0*	2.06*	100.0*	3.40*	100.0	6.67	94.5	10.42	36.7	41.67	100.0
	100.0*	3.96*	100.0*	6.53*	100.0	12.79	87.3	20.01	3.3	80.01	—
Sea water pH 2.7 (−11.0 m.v.)	100.0	4.04	98.6	6.66	100.0	13.05	89.7	20.42	83.3	81.67	100.0
	—	—	97.2	11.22	100.0	21.98	89.7	34.39	73.3	147.51	—

*, omitted from figure. {, one lot of eggs exposed to two different current strengths.

There are unmistakable differences between the adhesiveness in pH 2.7 and pH 4.7 and again between that in pH 4.7 and in the two higher pH values. But the difference in adhesiveness between the two higher pH values is hardly perceptible. This is caused by the fact that when the absolute magnitude of the potential increases well above the critical potential, the cells become so non-adherent that the present

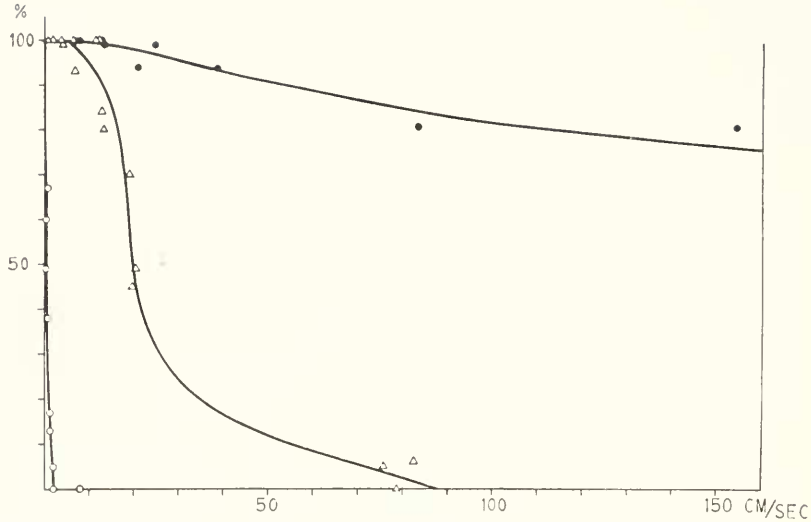

FIGURE 2 A. The remainder-curves of *Anthocidaris* eggs in sea water of pH 8.2 (normal sea water) (○ −○), pH 4.7 (△ − △) and pH 2.7 (● − ●). The ordinates are the percentages of the remaining cells, and the abscissae are the current strengths. The area circumscribed by a remainder-curve and the two axes is taken as the index of adhesiveness.

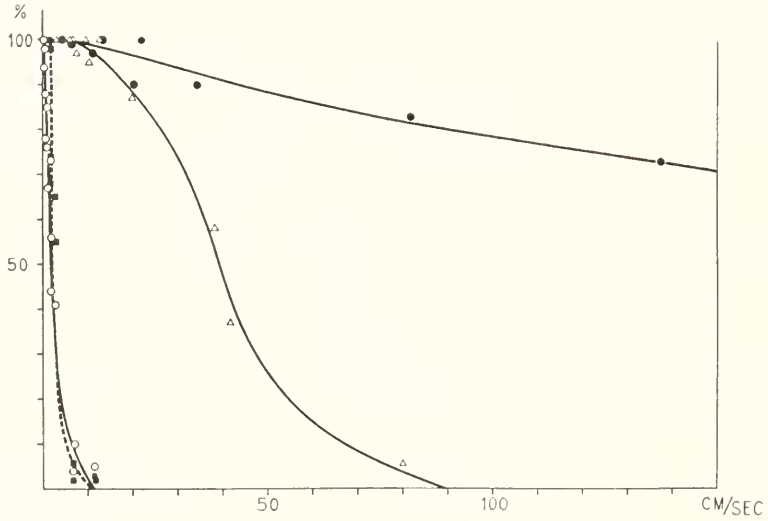

FIGURE 2 B. Another set of *Anthocidaris* remainder-curves in sea water of pH 8.2 (○ − ○), pH 6.9 (■ − ■), pH 4.7 (△ − △) and pH 2.7 (● − ●), showing the reproducibility of the results among different batches. Note also that the adhesiveness in pH 8.2 and that in 6.9 are nearly the same although they can be well differentiated by the gravity method (see Table I B).

technique is too crude to show the difference. Fortunately, however, the difference can be caught by the gravity method. On inverting the container, the number of the cells which fall is greater for pH 6.9 than for pH 8.2 on many occasions (see Table 1 B). In pH 6, the inversion of the tube fails to tear off any eggs from the wall, which indicates that at pH 6, the adhesiveness suddenly increases. It is extremely interesting to remember here that the absolute magnitude of the surface potential in *Anthocidaris* eggs suddenly decreases between pH 7 and pH 6.

It was pointed out previously that the change in cataphoretic potential caused by the change in hydrogen ion concentration is always perfectly reversible. The change in adhesiveness is also reversible. An example is given in Table 2 and Figure 3. The curves for before and after the treatment with the medium of pH 4.7 overlap perfectly. One batch of eggs was met with which became unusually adhesive in the medium of pH 4.7, but even in that case, the reversibility was perfect.

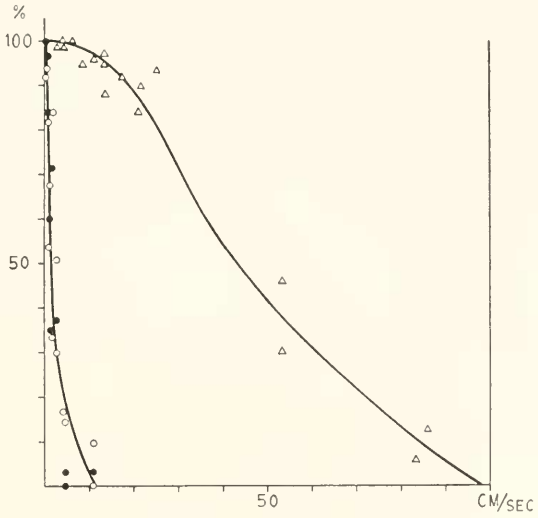

FIGURE 3. A set of records showing the perfect reversibility in the adhesiveness of the cell membrane of *Anthocidaris* eggs. ○ — ○, the remainder-curve in sea water of pH 8.2. △ — △, that in sea water of pH 4.7. ● — ●, that in sea water of pH 8.2 after the treatment with acidified sea water of pH 4.7.

Cerium ions and adhesiveness. The results of the cataphoretic measurements of a Na-Ce series were taken from the preceding paper (Dan, 1947b). These cataphoretic measurements were not made under exactly the same conditions as the present adhesiveness measurements, but they are believed to be comparable.

An example of the adhesiveness measurements in a Na-Ce series is given in Table 3 and Figure 4. This figure is self-explanatory. When the cerium concentration becomes higher, i.e., as the absolute magnitude of the negative potential decreases, the remainder curves become higher and higher and after passing 1/200 M $CeCl_3$, which is nearly the isoelectric concentration, the curves come down again. A comparison of the cataphoretic values with the adhesiveness values is given in

TABLE 2

Medium	%	cm./sec.	%	cm./sec.	%	cm./sec.	%	cm./sec.	%	cm./sec.
Sea water pH 8.2 (−23.0 m.v.)	94.0*	0.08*	91.9	0.14	98.8*	0.27*	81.7*	0.42*	33.3	1.67
	94.0*	0.24*	90.0*	0.39*	95.0*	0.77*	67.5	1.20	14.3	4.79
	94.0	0.55	81.8	0.90	84.0	1.77	50.8	2.76	9.5	11.04
	100.0*	0.21*	100.0*	0.34	84.3*	0.67*	53.7	1.04	16.7	4.17
	100.0*	0.53*	96.2*	0.88	78.1*	1.73*	29.9	2.71	0	10.83
Sea water pH 4.7 (−18.5 m.v.)	100.0*	0.55*	100.0*	0.90*	100.0*	1.77*	98.5	2.76	95.8	11.04
	100.0*	0.85*	100.0*	1.41*	98.3*	2.77*	98.5	4.32	91.7	17.29
	100.0*	2.63*	93.2*	4.35*	96.6*	8.53*	97.1	13.33	45.8	53.33
	100.0*	4.24*	93.2*	7.01*	96.6*	13.73*	89.7	21.46	12.5	85.83
	100.0*	1.23*	100.0*	2.04*	100.0	4.00	100.0	6.25	93.3	25.00
	100.0*	2.63*	100.0*	4.36*	94.7	7.53	87.8	13.33	30.0	53.33
	91.7*	4.12*	96.3*	6.80*	94.7	13.33	83.8	20.83	6.7	83.33
Sea water pH 8.2 (−23.0 m.v.)	90.2*	0.24*	94.4*	0.39*	84.2	0.77	35.0	1.20	0	4.79
	100.0*	0.23*	100.0	0.37·	82.1*	0.73*	60.0	1.15	3.1	4.58
	98.2*	0.55*	96.8	0.90	71.4	1.77	37.1	2.76	3.1	11.04

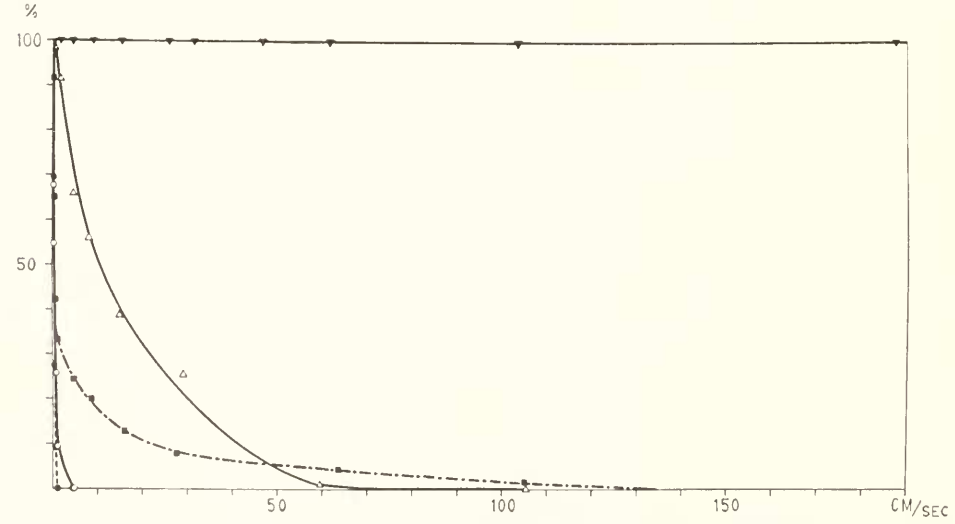

FIGURE 4. The remainder-curves of *Strongylocentrotus* eggs in 1/2 M NaCl (○ − ○), 1/3000 M $CeCl_3$ + NaCl (△ − △), 1/200 M $CeCl_3$ + NaCl (▼ − ▼), 1/50 M $CeCl_3$ + NaCl (■ − ■) and in 1/10 M $CeCl_3$ + NaCl (● − ●).

TABLE 3

Medium	%	cm./sec.	%	cm./sec.	%	cm./sec.	%	cm./sec.	%	cm./sec.
1/2 M NaCl (−39.7 m.v.)	67.7	0.24	54.7	0.39	5.7	0.77	9.4	1.20	0	4.79
1/3000 M $CeCl_3$ in NaCl	97.7	0.57	91.2	1.86	66.0	4.61	56.0	8.13	38.9	15.06
(−22.8 m.v.)	25.5	29.19	0.9	59.55	0	105.42				
1/200 M $CeCl_3$ in NaCl	100.0	1.79	100.0	4.56	100.0	9.11	100.0	15.57	100.0	25.89
(+4.0 m.v.)	100.0	31.53	100.0	46.88	100.0	61.67	100.0	103.54	100.0	187.50
1/50 M $CeCl_3$ in NaCl	91.7	0.24	65.4	0.40	42.2	0.76	33.3	1.22	24.4	4.69
(+21.4 m.v.)	19.9	8.69	12.8	15.91	8.0	27.71	4.5	63.75	1.7	105.00
1/10 M $CeCl_3$ in NaCl (+33.9 m.v.)	69.5	0.23	28.0	0.38	9.2	0.74	0	1.15	0	4.61

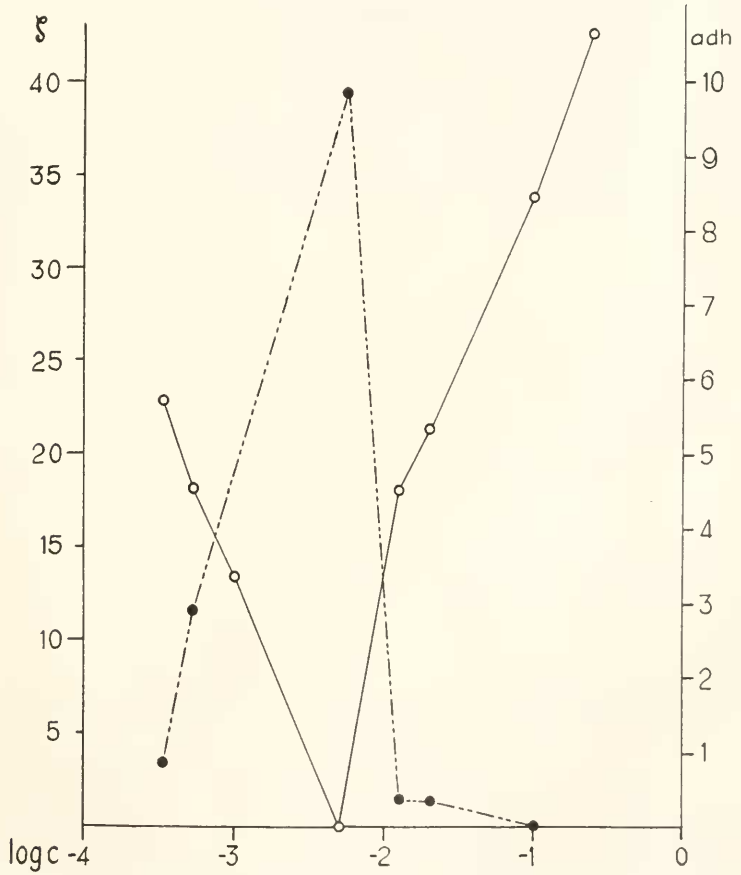

FIGURE 5. Inverse relation between the absolute magnitude of the zeta potential (○ − ○) and the adhesiveness (● −··− ●) as plotted against log c of cerium ions.

Table 4 and is represented graphically in Figure 5. Inverse correlation between the two properties is unmistakable. In this case, considering the crudeness of the technique, the adhesiveness values were roughly obtained by cutting out the plotted areas and weighing them instead of measuring the areas by a planimeter. The ratios of the adhesiveness values are in the order given in Table 4: e.g., 1 : 23.5 : 80.5 : ⟩⟩⟩ 273.6 : 10.2 ; 9.3 : 0.7.

TABLE 4

Conc. of Ce-ions in mol.	O(NaCl)	1/3000	1/2000	1/1000	1/200	1/80	1/50	1/10	1/4
Zeta potentials in m.v.	−39.7	−22.8*	−18.1*	−13.4	+4.0*	+18.1	+21.4	+33.9	+42.6
Ratios of adhesiveness values	1.0	23.5	80.5	—	273.6	10.2	9.3	0.7	—

* Potential values found by intrapolation.

SPECIFIC EFFECT OF DIFFERENT IONS ON ADHESIVENESS

If the catophoretic potential is the only factor involved in determining the adhesiveness of the cell membrane, the eggs should have the same adhesiveness at the same potential, no matter by which ions the change is brought about. In order to test this, 1/3 M $CaCl_2$, 1/2000 M and 1/80 M $CeCl_3$ in NaCl were selected and the adhesiveness in these solutions was tested. In all three solutions, the absolute magnitude of the potential is approximately 18 millivolts. The adhesiveness data are given in Table 5 and Figure 6. The figure shows that the above supposition is not true. Although the remainder curves in 1/3 M $CaCl_2$ and 1/80 M $CeCl_3$ follow a more or less similar course, that in 1/2000 M $CeCl_3$ runs decidedly higher. Of

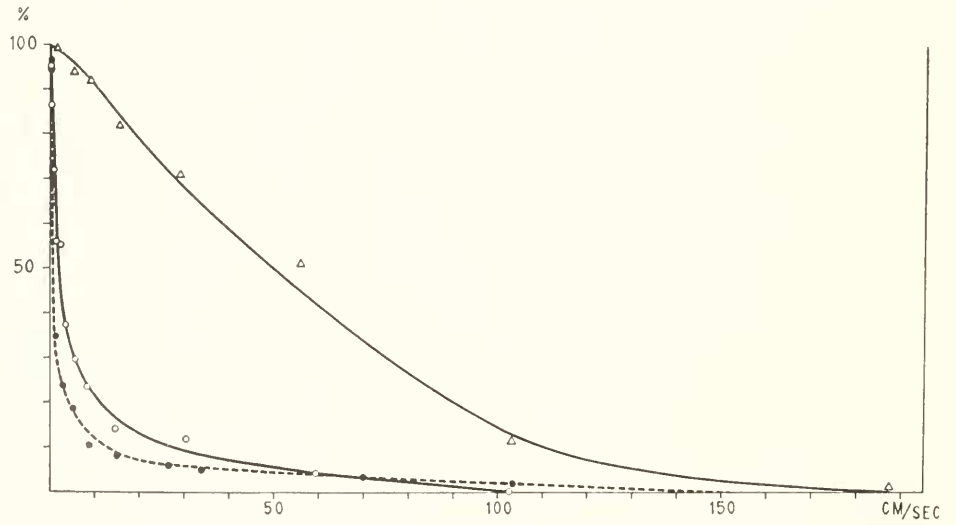

FIGURE 6. Comparison of the membrane adhesiveness of *Strongylocentrotus* eggs in three different media, in each of which the absolute magnitude of the cataphoretic potential of the egg surface is about 18 millivolts. ○ − ○, 1/3 M $CaCl_2$; △ − △, 1/2000 M $CeCl_3$ + NaCl and ● ····· ●, 1/80 M $CeCl_3$ + NaCl.

course, there is still room for the argument that since 1/2000 M cerium corresponds to the steepest part of the concentration-potential curve (see Dan, 1947b, Fig. 1), the greater adhesiveness here obtained may be due to a slight deviation of the cerium concentration to the more concentrated side. But a study of the acid series definitely negates this possibility. In a preliminary measurement, at pH 3 where the zeta potential of *Strongylocentrotus* eggs is − 19.6 millivolts, the adhesiveness turns out to be much higher than that in 1/2000 M $CeCl_3$.

TABLE 5

Medium	%	cm./sec.	%	cm./sec.	%	cm./sec.	%	cm./sec.	%	cm./sec.
1/3 M $CaCl_2$	95.1	0.26	86.5	0.44	71.9	0.81	55.9	1.33	55.4	2.39
(−17.5 m.v.)	37.4	3.58	29.5	5.58	23.3	8.25	14.0	14.60	12.0	30.42
	4.1	59.37	0	102.50						
1/2000 M $CaCl_3$	99.3	1.74	93.9	5.40	91.9	9.20	81.8	15.58	70.9	29.22
(−18.1 m.v.)	51.0	56.01	11.5	103.15	1.1	187.50				
1/80 M $CeCl_3$	94.3	0.25	96.3	0.41	68.5	0.77	34.6	1.28	23.3	2.92
(+17.2 m.v.)	18.5	5.10	10.4	8.80	8.1	15.01	6.1	26.53	5.0	33.75
	3.3	70.21	1.8	103.33						

The above finding is not surprising considering the fact that Northrop and De Kruif (1922a) came across the same sort of phenomenon. They conclude that within 0.01 to 0.1 N of a salt solution, the potential is a primary factor for adhesiveness, but above 0.1 N, the specific effect of individual ions begins to modify the general issue.

Another complication to be remembered is a biological factor. Reports can be found in the literature to the effect that some cells normally have no detectable potential yet are not sticky at all (Mudd, 1933). Among marine ova, *Cerebratulus* eggs belong to this class (Dan, 1934). Besides this, adhesiveness measurements show that immature eggs with germinal vesicles are stickier than ripe ones, with no difference in their potentials. In bacteriology, this secondary modification of the potential-adhesiveness relation is attributed to the state of hydration of the surface. At present, no evidence is available whether or not such a situation exists among marine ova.

At any rate, it can be concluded that so far as sea-urchin eggs are concerned, the cataphoretic potential is a predominant factor in determining stickiness in the majority of the cases, and that only under certain circumstances can biological factors and ionic conditions modify the general trend to a lesser extent.

DISCUSSION

In the preceding paragraphs, evidences have been put forward to show that the adhesiveness of the cell membrane of sea-urchin eggs is primarily determined by the electrical condition of the membrane.

When the zeta potential is shifted, the adhesiveness also changes. Therefore, it is evident that referring only to the critical potentials of flocculation is not a satisfactory method. This shortcoming can be supplemented by the present technique.

However, for the sake of comparison, if the critical potential of *Strongylocentrotus* eggs is suggested, it must lie in the neighborhood of ± 16 millivolts, because in the cerium series, − 13 millivolts is decidedly below the critical point and − 22 and + 21 millivolts are above it, while in the acid series, − 19 millivolts is just above the critical point. This figure of 16 millivolts agrees very well with the other data obtained in a variety of materials.

From the standpoint of cell physiology, two points of particular interest will be commented upon. When the correlation between the abolition of the surface potential and the agglutination of cells was established beyond any dispute in bacteria and erythrocytes, it naturally led investigators to attempt to discover the same relation in leucocytes. However, the careful investigations of Fauré-Fremiet (1927a, 1927b, 1928) and Fenn (1922) revealed the fact that the situation is either very complicated or the correlation fails entirely to hold in the leucocytes of both vertebrates and invertebrates. This fact may be taken as very instructive. As is well known, bacteria and erythrocytes are comparatively inert cells, and as such, are predominantly fitted for the analysis of purely physico-chemical factors. Attempts for further advancements along this path might very likely have been attended with more success if they had been directed to egg cells rather than to leucocytes. The present work will offer a good example to bring out the contrast of the two materials.

The last question is how far the cataphoretic potential is connected with vital activity. In the present stage of our knowledge, no unanimity has been reached among investigators. Many of them even negate a possible correlation between the two. Yet, some positive evidences are accumulating. In the bacteriological field, it is often suggested that the virulence of pathogenic bacteria (Rosenow, 1933a, 1933b, 1934; Rosenow and Jensen, 1933) and the nitrogen fixing power of soil bacteria (Tittsler, Lisse and Ferguson, 1932) are correlated with the surface potential.

As for sea-urchin eggs, the data are too scanty to warrant a definite conclusion. But it is interesting to point out that Schechter (1937), in his study on the endurance of fertilizability of *Arbacia* eggs in mixtures of NaCl and $CaCl_2$ in various ratios, found that the eggs remained fertilizable longest in straight NaCl. This means at least in this case, that the longest viability is associated with the highest magnitude of the zeta potential. (In *Arbacia,* the dissolution of the calcium compound complicates the situation, but so far as the underlying surface is concerned, this statement holds. See Dan, 1936, Table II.)

This interdependence of high negativity and longevity of the eggs has an interesting connection with the electrokinetic conditions prevailing on the egg surface in a normal environment. In *Arbacia,* the potential on the internal granules is ca. − 10 millivolts, that of the protoplasmic surface is ca. − 20 millivolts, that of the calcium compound is ca. − 30 millivolts (Dan, 1936) and finally the potential of the jelly is ca. − 35 millivolts (Dan, 1933). In other words, the zeta potentials of the enveloping layers become higher in absolute magnitude in the order from the interior to the exterior. Among the data so far collected by the author, there is not a single exception to this arrangement (Dan, 1934). A priori thinking, where there is a barrier of a high zeta potential, more energy will be required for charged substances to pass through it, for substances carrying a charge the same in sign as that of the barrier will be repulsed at the boundary, while substances with the opposite sign will be tightly adsorbed. Thus preventing an unnecessary exchange of

substances with the environment, this condition may be effective in the protection of the cell interior. This is particularly significant for egg cells which have enough stored nutrient materials to live for a while independent of their surroundings. If we are allowed to go one step further in imagination, this fact may have something to do with the unexpectedly high values in the potentials of egg cells. But until further researches are made, this shielding effect by the high potential of the enveloping layer is simply offered as a suggestion.

Summary

1. A simple apparatus is described for measuring the adhesiveness of the cell membranes of sea-urchin eggs by the stream method.

2. By constructing a curve of the percentages of remaining cells in different current strengths (remainder curve), adhesiveness is expressed by the area circumscribed by the curve and the two axes.

3. In *Anthocidaris* eggs, when the zeta potential is changed by hydrogen ions, the adhesiveness changes correlatively and the shifts in the potential and the adhesiveness are both perfectly reversible.

4. In *Strongylocentrotus pulcherrimus*, both the potential and the adhesiveness change pari pasu when cerium ions are added to the medium. Adhesiveness is maximum at the isoelectric point.

5. Specific effects of individual ions play a secondary role in determining the membrane adhesiveness.

6. Related phenomena are discussed.

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